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Brief Research Communication

A Study of Genetic Association Between Manic-Depressive Illness and a Highly Polymorphic Marker From the GABRβ-1 Gene

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We report on an association study between a tetranucleotide repeat polymorphism in the GABRβ1 gene and manic-depressive illness in a Spanish population. This gene may be an important candidate for bipolar affective disorders since severe GABergic alterations have been described in patients. Although our results do not reveal a clear evidence for association between manic-depressive illness and GABRβ1, we have found significant differences between patients and controls in the female subpopulation. Am. J. Med. Genet. 74:342–344, 1997.

KEY WORDS: GABA; manic-depression; genetics; association; affective disorder; GABRβ-1; bipolar; gene locus

INTRODUCTION

Manic-depressive illness (MD) is a major psychiatric disorder characterized by severe mood changes and a variable age of onset. Genetic factors have long been implicated in the etiology of MD, but little is known about the mode of inheritance and the specific genes involved. Linkage analysis have claimed both, X-linked [Baron et al., 1987; Mendlewicz et al., 1897] or autosomal dominant [Egeland et al., 1987] inheritance. However, initial reports suggesting major genes have not been reproduced [Baron et al., 1993; Kelsoe et al., 1989]. This failure may be related to the complexities inherent to an illness such as MD, including incomplete penetrance, phenocopies, gene-environment interaction, the possibility of multiple disease susceptibility loci, as well as epistatic interaction between two or more loci and diagnostic uncertainties.

Population-based association studies with candidate genes, in which the frequency of an allele at the marker locus in controls is compared with that of patients, may be very useful as a complement to linkage analysis. This strategy have been successfully performed in a number of multifactorial disorders [Hodge, 1993].

Gamma-aminobutiric acid (GABA) is one of the major inhibitory neurotransmitters in the mammalian central nervous system and may be involved in the pathophysiolgy of MD for several reasons. First, the concentration of GABA is known to be decreased in cerebrospinal fluid (CSF) and plasma in both the manic and the depressed phase of bipolar patients [Petty et al., 1993]. Second, there exist some GABA agonists which are effective in the treatment of affective disorders. Third, several authors have described severe GABAergic alterations in manic-depressive patients [Squires et al., 1991].

In an association study, an appropriate candidate gene is of utmost importance. We have searched for a possible association between MD and a tetranucleotide (GATA)n repeat polymorphism previously described in GABRβ1 gene [Dean et al., 1991] in a Spanish population which had not been previously studied. This gene is located on chromosome 4 (4p12-13) [Schofield et al., 1989] close to the $GABRA_A\alpha 2$ gene. The $GABA_A$ receptor presents a multimeric structure composed by subunits of different types, and $\alpha 1\beta 2\gamma 2$ seems to be the most frequent type of $GABA_A$ receptor, thus, $\alpha 2$ and $\beta 1$ subunits may be present in $GABA_A$ receptors that appear only on a small minority of neurons, and hence it is not implausible that some alteration in them could be pathogenic but not lethal. In addition, $\beta 1$ subunit is known to be expressed on hippocampus [Costa, 1992], a region involved in the control of emotivity.

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MATERIALS AND METHODS

Subjects

We studied a total of 55 biologically unrelated patients (33 females and 22 males) diagnosed as bipolar I (43 subjects, 22 females and 18 males) or bipolar II (16 subjects, 11 females and 4 males) and 69 phenotipically normal controls (35 females and 34 males). All subjects were older than 40, ethnically Caucasians, and lived in Central Spain. Diagnoses was made by multiple interviews of patients using the Research Diagnosis Criteria (RDC). Re-evaluations were made by other psychiatrists blind to previous diagnoses or treatment response.

Genotyping

Genomic DNA was extracted from 20 ml venous blood samples using a simplified method (GNOME DNA Kit from BIO 101). Tetranucleotide repeat polymorphism (GATA)n at the GABRβ1 gene has been studied by PCR amplification using the primers described in Dean et al. [1991]. DNA samples (250 ng) were amplified in a volume of 50 μl with a final concentration of 10 mM Tris-Cl, pH 8.4, 50 mM KCL, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 10 μM of each primer, and 2 units of DynaZyme polymerase (Finnzymes Inc., Finland).

PCR samples (10 μl) were resolved by electrophoresis on 12% non-denaturing acrylamide (T12/C3) gels, and detected by silver staining (Bio-Rad Silver Stain Kit, BIO-RAD). Fragment sizes were measured relative to size standards.

The statistical significance of the null hypothesis of random association between GABRβ-1 polymorphism and manic-depressive illness was tested by usual chi-square statistic in contingency tables.

RESULTS AND DISCUSSION

A total of seven alleles differing in the number of GATA repeats at the GABRβ-1 locus have been detected (Figs. 1, 2). Genotype frequencies of the marker locus in controls and patients, respectively, were not statistically different from Hardy-Weinberg expectations.

Figure 2 shows the allele frequencies in male, female, and total population for both Control and Bipolar groups. As allelic distribution seem to be different between females and males the study of genetic association has been carried out separately for each sex as well as in the total population. We have examined the association for each allele of GABRβ-1 vs. the remaining alleles pooled and MD (Table I). The 2×2 contingency tables seem to indicate a significant association between the 154 bp allele and MD in females. Curiously, the 146 bp allele is more frequent in controls than in manic-depressive population.

In spite of this, significant differences have not been observed when 7×2 contingency tables have been obtained. This result may indicate that the positive asso-

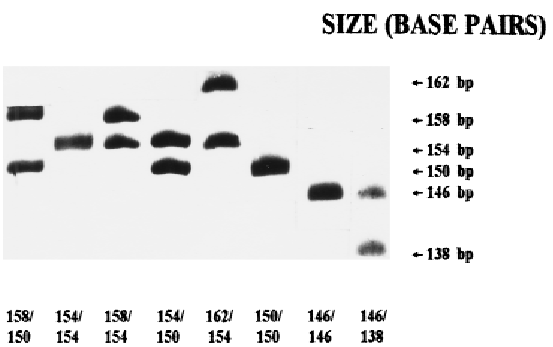

Fig. 1. A non-denaturing silver stained gel, showing alleles at GABRβ1 locus.

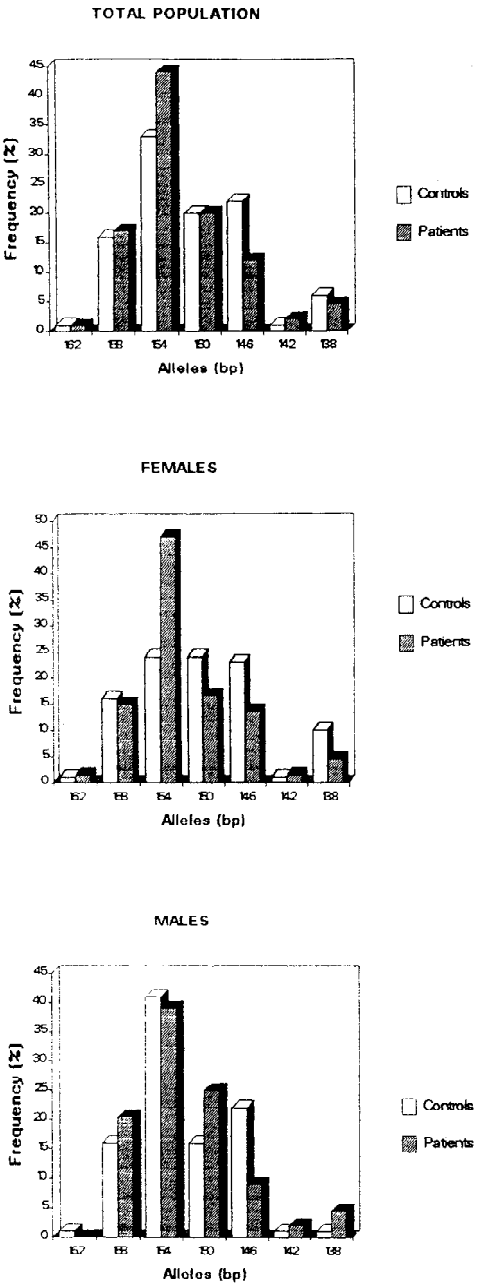

Fig. 2. Allele frequency in general population; females and males considered separately.

TABLE I. GAB-1 vs. Remaining Alleles Pooled and χ^2 MD

1 allele versus others	Females	Males	Totals
162/others	0	0.65	0.15
158/others	0.01	0.33	0.08
154/others	**7.97***	0.07	3.62
150/others	1.21	1.32	0
146/others	1.82	2.34	**4.20***
142/others	0	0.10	0.05
138/others	0.79	0.09	0.19

*$P < 0.05$.

ciation found between the 154 allele and manic-depressive illness in females could be spurious. In fact, when adjustment of the significance level for multiple test is performed by the Bonferroni method, there is no evidence for such association between 154 allele and MD.

However, adjustment of the significance level reduces the type I error for null associations, whereas it increases the type II error for those associations that are not null, making the test highly conservative. The increment in the type II error is expected to be especially adverse for the detection of weak associations. Even a general policy of not making adjustment for multiple comparisons has recently been suggested [Rothman, 1990]. Then, our results may be interpreted in a different way, and it may be a weak association between DNA polymorphism at the GABRβ-1 gene and MD that would require a very large population size to be statistically detected in the 7×2 contingency table.

Thus, although our results do no provide clear evidence for association between GABRβ-1 locus and MD in Centre Spain, the tendency observed with the 154 allele in females allows us to propose GABRβ1 gene as a good candidate for subsequent studies about the genetic basis of manic-depressive illness.

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